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Previews

GABA Is an Endogenous Ligand for Synaptic Glycine Receptors

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Experimental evidence refuting Dale's principle, the notion that each neuron synthesizes and releases only one neurotransmitter, has accumulated in the past four decades, and cotransmission by multiple neurotransmitters from the same axon terminal (and even from the same vesicle) now is well documented. Heretofore, in all examples of cotransmission, each released neurotransmitter acted on a different receptor. Lu, Rubio, and Trussell, however, demonstrate in this issue of *Neuron* the first instance of cotransmission in which two neurotransmitters act on the same postsynaptic receptor.

That a single neuron can make use of multiple neurotransmitters was controversial when first proposed (Burnstock, 1976), but now is well accepted (Seal and Edwards, 2006). Though this phenomenon (called cotransmission) initially was thought to be limited to neuromodulatory systems (e.g., release of neuropeptides from monoaminergic neurons; Hokfelt et al., 2000), cotransmission involving the "fast" neurotransmitters acetylcholine, glutamate, GABA, and glycine has been observed at a number of central synapses (Seal and Edwards, 2006). The best-studied example of cotransmission is the corelease of GABA and glycine in the spinal cord and brainstem, documented first by Jonas et al. (1998) and thereafter by a number of other investigators.

The work of Jonas et al. (1998) validated a hypothesis of GABAergic and glycinergic cotransmission based on immunohistochemical and biochemical studies: at some inhibitory synapses in the brainstem and spinal cord, GABA and glycine are colocalized presynaptically in nerve terminals, in which they are substrates for the same vesicular transporter, and $GABA_AR_s$ and GlyRs are colocalized postsynaptically (Burger et al., 1991; Todd et al., 1996). By recording individual quantal, miniature inhibitory postsynaptic currents (mIPSCs) mediated by both $GABA_A$Rs and GlyRs, Jonas et al. (1998) and others confirmed that $GABA_A$ and GlyRs colocalized at the same synapses are activated by GABA and glycine released from the same vesicle. This cotransmission by GABA and glycine likely permits the time course of postsynaptic inhibition at single synapses to be varied over a wide range, as $GABA_A$R-mediated synaptic currents are slower than GlyR-mediated ones (e.g., Jonas et al., 1998).

Importantly, in previously described examples of cotransmission, each neurotransmitter acts on a different receptor at the same synapse (i.e., at the same synapse, GABA binds to GABARs and glycine binds to GlyRs). Here, Lu, Rubio, and Trussell (Lu et al., 2008) describe a new and unique variant of GABAergic and glycinergic cotransmission in which GABA and glycine act as coagonists at the same receptor (the GlyR). The joint action of GABA (a weak agonist) and glycine (a strong agonist) on the receptor accelerates its rate of deactivation, thereby accelerating the time course of the glycinergic synaptic conductance. This remarkable finding raises the possibility that the timing of synaptic inhibition throughout the CNS may be modulated by the neurotransmitter composition of individual vesicles.

Lu, Rubio, and Trussell examined glycinergic synaptic inputs to the rat medial nucleus of the trapezoid body (MNTB), an auditory brainstem nucleus that is part of the neural circuitry underlying sound localization (reviewed by Kandler and Gillespie, 2005). The MNTB receives excitatory input from the contralateral cochlear nucleus and sends an inhibitory (glycinergic) projection to the ipsilateral superior olivary complex, which receives excitatory input from the ipsilateral cochlear nucleus. By integrating ipsilateral excitatory inputs and contralateral inhibitory inputs, superior olivary neurons encode differences in intraural stimulus timing and intensity.

To permit small differences in intraural stimulus timing and intensity to be encoded, signal transfer between the auditory nuclei in the brainstem must be precise and reliable. Excitatory input to each MNTB neuron consists of a single calyceal terminal (from an axon of a neuron in the cochlear nucleus) containing hundreds of glutamatergic active zones; as a consequence, MNTB neurons exhibit large, fast excitatory synaptic conductances ($\sim$150 ns, rise time $\approx$150 μs, half-width $\approx$500 μs) that ensure that even high-frequency input from the cochlear nucleus is encoded in action potentials that are transmitted reproducibly to the superior olive (e.g., Taschenberger and von Gersdorff, 2000). Synaptic inhibition in the mature MNTB is equally fast and powerful: Awatramani et al. (2004) found that mature MNTB neurons exhibit a glycinergic conductance equivalent in magnitude and time course to their excitatory conductance and sufficient to shunt calyceal excitation and inhibit action potential generation. In fact, the observed time course of inhibition was so rapid that the investigators were compelled to comment: "…it seems likely that the [glycine] receptors in mature MNTB contain a novel subunit or posttranslational modification (Awatramani et al., 2004)."

In this manuscript, Lu, Rubio, and Trussell demonstrate that the rapid time course

of inhibition at glycinergic synapses onto MNTB neurons arises not from unique intrinsic properties of the postsynaptic GlyRs but rather from their behavior when both the weak agonist GABA and the strong agonist glycine are bound jointly during GABAergic and glycinergic cotransmission. The path by which this conclusion is reached is an interesting one, and two observations appear to have directed the authors toward it. One, although developmental shifts from inhibitory cotransmission to pure glycinergic inhibition have been documented in the auditory brainstem, it is uncertain whether these reflect a loss of presynaptic GABA, postsynaptic $GABA_A$Rs, or both (Kandler and Gillespie, 2005). Two, the decay of GlyR-mediated macroscopic currents evoked by rapid application of glycine to outside-out patches excised from MNTB neurons is much slower than the decay of GlyR-mediated synaptic currents recorded in those same neurons (Lu et al., 2008).

Lu, Rubio, and Trussell postulated that were GABA to be released from mature glycinergic terminals presynaptic to MNTB neurons, it would affect the behavior of postsynaptic GlyRs. Thus, the mismatch between the observed time courses of GlyR-mediated patch and synaptic currents reflects the fact that glycine is applied alone to elicit patch currents, but GABA and glycine are coagonists of GlyRs during synaptic transmission. It was relatively straightforward for the authors to demonstrate that coapplication of GABA and glycine accelerates GlyR-mediated macroscopic patch currents, making them resemble more closely mIPSCs (Figures 1C–1E in Lu et al., 2008) and to prove by electron microscopic immunohistochemistry that GABA and glycine are colocalized in inhibitory axon terminals presynaptic to MNTB neurons (Figures 4, S2, and S3 in Lu et al., 2008). How, though, could Lu, Rubio, and Trussell prove that synaptically released GABA and glycine act on the same GlyRs on MNTB neurons?

One possible line of experimentation would make use of genetically altered mice lacking the enzyme that synthesizes GABA from glutamate: glutamic acid decarboxylase (GAD; reviewed by Soghomonian and Martin, 1998). There are two GAD isoforms, GAD_{65} and GAD_{67}; GAD_{67} knockout mice die at birth; GAD_{65} mice are viable, but exhibit altered activity-dependent plasticity in sensory (visual) cortex as well as other neurological deficits (Hensch, 2005). Given that postnatal development of auditory brainstem microcircuits is activity dependent (Kandler and Gillespie, 2005), the GAD_{65} knockout mouse is not an optimal model for the study of GABAergic and glycinergic cotransmission to mature MNTB neurons.

Fortunately, a simple pharmacological manipulation can reduce vesicular GABA concentration. Because GABAergic nerve terminals possess neuronal glutamate transporters to provide GAD with substrate, pharmacological blockade of these transporters inhibits GABAergic synaptic transmission. Lu, Rubio, and Trussell reasoned that blocking glutamate uptake into inhibitory terminals would reduce the GABAergic component of cotransmission, thereby reducing the effect of GABA on GlyRs and slowing mIPSCs. They were correct, as illustrated in Figure 6 of their article, and their observation was confirmed by blocking the effects of glutamate uptake inhibitors with a GAD inhibitor (which also reduced the GABAergic component of cotransmission).

In summary, Lu, Rubio, and Trussell have demonstrated quite convincingly that cotransmission by GABA and glycine shortens the time course of GlyR-mediated synaptic conductances in the MNTB. Why, though, is GABA necessary to accelerate glycinergic transmission? The answer likely lies in the fact that relative to $GABA_A$Rs, GlyRs are limited in their kinetic range. Dozens of distinct $GABA_A$Rs, composed of pentameric combinations of multiple subunits, exist in the mammalian nervous system, and they differ widely in their kinetics and ability to be modulated (Mody and Pearce, 2004). GlyRs, however, are significantly less diverse: in the mature mammalian nervous system, most GlyRs are thought to be heteromeric pentamers comprising a combination of one of two α subunits (α1 or 3, with α1 being by far the most common) and one β subunit (Lynch, 2004). Because GlyR composition is not a variable that can be changed significantly from synapse to synapse, cotransmission by GABA and glycine presents a flexible method by which the time course of glycinergic transmission can be adjusted to match the requirements of different neural circuits.

The findings of Lu, Rubio, and Trussell raise the possibility that GlyRs are modified by GABAergic cotransmission at other synapses in the central nervous system in which both GABA and glycine are found presynaptically. Apart from the auditory brainstem, in which many inhibitory neurons produce both neurotransmitters, an interesting example of cotransmission is found in the cerebellum: Golgi cells contain GABA and glycine and contact both granule cells and unipolar brush cells (Dugue et al., 2005). Golgi-granule cell transmission appears to be mediated solely by $GABA_A$Rs and Golgi-unipolar brush cell transmission by either $GABA_A$Rs or GlyRs, but not both in an individual neuron (Dugue et al., 2005). Perhaps the GlyRs on unipolar brush cells bind GABA as well as glycine? Further, it is interesting to consider the possibility that glycine may act as a coagonist at some $GABA_A$Rs.

This study by Lu, Rubio, and Trussell also serves to answer a question that has been in my mind for years. When I studied the relationship between GlyR channel kinetics and the time course of glycinergic synaptic transmission to hypoglossal motoneurons as a graduate student, I could not understand why the macroscopic GlyR patch currents that I recorded were slower than glycinergic mIPSCs, no matter what experimental manipulation the patch currents were subjected to. The irony of this is that while I was doing my thesis work, another graduate student in the laboratory, Jennifer O'Brien, was documenting GABAergic and glycinergic cotransmission to hypoglossal motoneurons! If only we had thought to combine our projects!

Of course, a hallmark of a great scientific study is the ability to approach an established problem from a fresh perspective, and certainly the present work by Lu, Rubio, and Trussell characterizes this. Who would have thought that GABA is an endogenous ligand for GlyRs? Not me, unfortunately.

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Ama"Zinc" Link between TrkB Transactivation and Synaptic Plasticity

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While Trk receptors can be activated in a neurotrophin-independent manner through "transactivation" by GPCR ligands, its physiological significance in the brain remains unknown. Huang et al. have now identified a novel mechanism of TrkB transactivation. They show that zinc ions can transactivate TrkB independent of neurotrophins and that such a transactivation is important for mossy fiber long-term potentiation (LTP).

A dogma in the neurotrophin field, and indeed in cell signaling in general, is that neurotrophins signal by binding their cognate Trk receptors at the cell surface. Ligand binding initiates Trk receptor dimerization and phosphorylation via its intrinsic tyrosine kinase. Phosphorylated intracellular cytoplasmic tails of Trk act as docking sites for adaptor molecules, which in turn activate any/all of the three major downstream signaling pathways (Ras/MAPK/Erk, PI3K/Akt, and PLC-γ) to regulate neuronal survival, neurite outgrowth and branching, and synaptic transmission and plasticity (Huang and Reichardt, 2003). Until recently, Trk receptors were believed to be activated solely by neurotrophins. For example, TrkB is activated by either brain-derived neurotrophic factor (BDNF) or neurotrophin 4 (NT4). A neurotrophin-independent mechanism for TrkB activation was implicated by the observation that neither a null mutation of NT4 nor a conditional deletion of BDNF prevented TrkB activation during epileptogenesis (He et al., 2006, 2004). Intriguing studies by Lee et al. (Lee and Chao, 2001; Lee et al., 2002) provided evidence that, at least in cell culture, Trk signaling can occur independently of direct ligand/neurotrophin binding through "transactivation" by G protein-coupled receptor (GPCR) ligands such as adenosine or the neuropeptide PACAP. As the term suggests, transactivation refers to ligand-independent, indirect activation of receptor signaling. Further studies demonstrated that Trk receptors are activated intracellularly through Src kinase-mediated tyrosine phosphorylation. However, it was unclear whether molecules other than GPCR ligands can transactivate Trks. Moreover, the physiological significance of Trk receptor transactivation in the brain in vivo was also difficult to ascertain. The study by Huang et al. published in this issue of *Neuron* represents a major leap forward: the divalent cation zinc released in the hippocampal CA3 area in response to neural activity not only transactivates TrkB in vivo, but this transactivation plays a significant role in long-term potentiation (LTP) at the mossy fiber (MF)-CA3 synapses (Huang et al., 2008).

Previous studies have suggested that zinc (and copper) can activate TrkB by converting proBDNF to mature BDNF (mBDNF) through extracellular metalloproteinases (Hwang et al., 2005, 2007). In the present study, Huang et al. performed a series of experiments to show that zinc can directly activate TrkB in the absence of BDNF. First, zinc, but not other divalent or monovalent cations (Mg^{2+}, Ca^{2+}, K^{+}, Na^{+}, etc.), selectively activates TrkB (not TrkA or TrkC) and its downstream signaling pathways (Erk1/2, CREB, and PLC-γ). Second, zinc transactivates TrkB not only in wild-type (+/+) neurons when extracellular BDNF has been removed by the scavenger TrkB-IgG, but also in neurons derived from BDNF null mutant (−/−) or in TrkB-expressing heterologous cells. Third, zinc-induced TrkB activation was